As mentioned before, Zhang and Macara previously found that Par3 regulates spine maturation by spatially restricting Tiam1 localization and Rac activation to dendritic spines, and this function is independent of its association with aPKC and Par6 (Zhang and Macara, 2006). They now find that the Par6/aPKC complex controls spine biogenesis and maintenance rather than spine maturation, and, intriguingly, that the underlying mechanism involves spatial regulation of RhoA activity (Figure 1B). Using a FRET biosensor, they show that Par6 inactivates RhoA in the spines. Based on previous studies, the authors considered the possibility of Smurf1 involvement in this process (Wang et al., 2003). However, Zhang and Macara do not detect any changes in RhoA protein levels when Par6 levels are altered in hippocampal neurons. Instead, they show that Par6/aPKC inactivates RhoA through a negative regulator of RhoA, p190A RhoGAP. Coexpression of a GAP-deficient p190A mutant with Par6 attenuates the Par6-induced increase in spine density, and, importantly, knockdown of p190A diminishes the Par6-triggered decrease in RhoA activity as measured by FRET analysis.

How Par6/aPKC regulates p190A remains unclear. Furthermore, the upstream inputs that regulate Par6/aPKC and Par3/Tiam1 in dendritic spines, and the role of these complexes in synaptic function, are also unknown. In spines, Par6/aPKC regulation seems to be independent of Cdc42 (Zhang and Macara, 2008). Interestingly, previous studies showed that Tiam1 interacts with the NMDA receptor and is phosphorylated following receptor activation (Tolias et al., 2005). Whether Par3 is somehow connected to the NMDA receptor is not known.

Together, the studies by Nakayama et al. and Zhang and Macara unveil novel links between members of the Par complex and the RhoA/ROCK signaling pathway, defining additional modes by which Rho GTPases interplay with Par complex components. In the establishment of front-rear polarity of migrating cells, RhoA/ROCK controls Par complex formation and activity and thereby (Cdc42-induced) Rac activation. In the context of spine morphogenesis, the Par6/aPKC module downregulates RhoA/ROCK activities. Whereas RhoA/ROCK proteins play different roles in the above cellular polarization events, both studies highlight that the establishment of cell polarity relies on the interplay between signaling by members of the Par complex and Rho GTPase family members.

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Taming the Tiger of Tissue Aggregation: How Epithelia Control Structural Assembly of Underlying Cells

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A recent paper by Ninomiya and Winklbauer explores the role of the epithelium in restricting the natural capacity of mesenchymal cells to form amorphous aggregates. They propose the epithelium controls mesoderm adhesion, thereby ensuring that embryos and, by extension, adults are not multilayered balls of cells, but are instead elongate with a linearly segmented architecture.

Morphogenesis involves the coordinated rearrangement of cells to produce tissues organized into both linear segments and laminar sheets. Cells are channeled into single-layered or double-layered sheets, whether they are epithelial or mesenchymal, with distinct anterior-posterior segmental identities. Whereas epithelial cells are confined to a single layer by their intrinsic apical-basal polarity, mesenchymal cells are organized into sheets defined by their association with other

germ layers or cell types and lack a well-defined apical-basal polarity (Green and Davidson, 2007). Exactly how early embryos coordinate epithelial and mesenchymal cell generation of self-supporting 3D structures still confounds embryologists. Previous work on interactions between the planar cell polarity pathway and fibronectin/integrin have shown that these systems mediate many of the cell behaviors operating within the mesoderm (Marsden and DeSimone, 2003); however, the current paper illustrates how even these basic pathways may be regulated by the epithelium that separates mesoderm from the archenteron, the primitive gut. Using microsurgical techniques first pioneered on amphibian embryos nearly 100 years ago, Ninomiya and Winklbauer (2008) expose how the tissue-sculpting processes of aggregation, elongation, and engulfment depend on the establishment of apical-basal polarity in the overlying epithelium.

Ninomiya and Winklbauer (2008) first challenge the instructional role of the epithelium on tissue shape and extension within naturally elongating frog embryonic tissues. Once isolated from their protective epithelial sheet, deep mesenchymal tissues ball up within minutes. Such rapid movements are many times faster than normal morphogenetic movements. Embryologists considered these movements part of a program of wound healing that has made amphibians a useful model system for experiments requiring microsurgery. One explanation of the rapidity of both wound healing and tissue aggregation is that exposure of embryonic mesenchymal cells to external media activates high levels of cell-cell adhesion in the exposed cells.

Considerable support for the role of differential cell adhesion in morphogenesis has come from both experimental and theoretical studies (Steinberg and Gilbert, 2004). Embryonic tissues can be empirically tested for their ability to engulf other tissues. For instance, a cellular aggregate of endoderm, when challenged by a mesodermal aggregate, will be engulfed by those cells, thus mimicking the natural alignment of endoderm-within-mesoderm germ layers after gastrulation. By wrapping an epithelial sheet around a rod-shaped mass of mesenchymal tissue, Ninomiya and Winklbauer (2008) show that the epithelium plays a key role in establishing a linearly segmented architecture by inhibiting rapid aggregation of the rod-shaped mesenchymal mass into a sphere. Without an epithelium, even the normally self-sculpting dorsal lip of the frog embryo rounds up into a featureless sphere. Providing prospective mesodermal aggregates with an epithelial "coat" restores their capacity to elongate. These experiments suggest two possibilities: epithelia might simply provide a conducive environment, or epithelia might pass along polarity cues that serve to inhibit aggregation in the underlying mesenchyme. In order to test the latter possibility, Ninomiya and Winklbauer (2008) perturb the apical-basal polarity in the epithelium by overexpressing lethal-giant larva (Lgl) and atypical protein kinase C (aPKC), key proteins in the PAR/Scribble/Discs Large pathway establishing apical basal-polarity (Goldstein and Macara, 2007). By recombining tissues expressing Lgl/aPKC, they demonstrate that Lgl/aPKC-mediated apical-basal polarity in the epithelium allows elongation of the mesoderm after contact between the two layers is established.

How might polarity cues inhibit aggregation and what role does Lgl/aPKC localization play in transmitting these cues to mesenchymal cells? Both qualitative and quantitative theoretical models based on surface tension have been used to explain tissue aggregation and engulfment. Ninomiya and Winklbauer (2008) propose that apical-basal polarity of the epithelial layer reduces surface-tension-like properties of the mesoderm. To test this Ninomiya and Winklbauer (2008) modulated cell-cell adhesion within the tissues by expressing a protocadherin, M-PAPC, which has been shown to increase cell mixing by reducing cadherin-mediated cell adhesion (Chen and Gumbiner, 2006). However, just like in physics, the mechanistic origin of surface tension is not always clear. The simplest explanation postulates that differences in cell-cell adhesion generate surface-tension-like properties which then drive early morphogenesis; however, other cellular properties such as cell motility, cortical contractility, and tissue stiffness also may generate surface-tension-like properties in embryonic tissues and are equally likely players in guiding tissue morphogenesis (Harris, 1976). This may be the case in Ninomiya and Winklbauer's studies since attempts to recapitulate differential cell-cell adhesion using M-PAPC fail to reproduce the effects of Lgl/aPKC on elongation.

Lgl/aPKC might guide underlying mesenchymal cells by acting via several pathways, including instructive signaling pathways, or by driving differential cell behaviors to reduce surface-tension-like properties in the mesoderm. Evidence for both possibilities can be found in cases where PAR complex mediates asymmetric cell division and the polarized delivery of differentiation factors to daughter cells. After early cleavage stages *Xenopus* embryonic epithelial cells divide in the plane to generate two equivalent epithelial daughter cells (Chalmers et al., 2005). After Lgl localization is disrupted, epithelial cells may also divide asymmetrically, often leaving one daughter cell in the epithelium and another daughter cell in the deep layer with mesenchymal properties. Ninomiya and Winklbauer (2008) also see abnormal asymmetric cell division after Lgl/aPKC is overexpressed. Disrupted delivery of inherited differentiation factors may indicate larger defects in the ability of these cells to direct their secretory apparatus (Wirtz-Peitz and Knoblich, 2006). Furthermore, by causing epithelial-derived mesenchymal cells to mix with the mesoderm, Lgl/aPKC might disrupt endogenous polarity processes operating within the mesoderm.

In addition to disrupting polarized cell behaviors such as cell division, overexpression of Lgl/aPKC might perturb cell contractility (Munro, 2006). Lgl and aPKC interact with myosin II and the cortical actin cytoskeleton. PAR-mediated interference with myosin II is known to disrupt both gastrulation in *C. elegans* and germ band elongation in *Drosophila* by reducing cell contractility. Lgl/aPKC activity within the epithelial layer may alter differential cell contractility, differential cell motility, or differential tissue stiffness, which can serve as alternative sources of surface-tension-like properties in embryonic tissues.

It is attractive to consider the effects of epithelial Lgl/aPKC alone on differential adhesion; however, a closer look at cell behaviors and cytoskeleton might reveal contributions of cell contractility and cell motility. Elucidating the physical and cellular mechanics of engulfment is a difficult problem in embryology, and the challenge

for future experiments will be to integrate the physical processes of cellular mechanics, cell motility, adhesion, and tissue architecture with the detailed workings of cellular biochemistry. The paper by Ninomiya and Winklbauer provides an excellent starting point by complementing molecular approaches with the powerful microsurgical techniques that brought amphibian model systems to the forefront of embryology.

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FERMing Up the Plasma Membrane

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As cells enter mitosis, shape changes occur that involve rearrangements of the actin cytoskeleton and an increase in cortical stiffness. In a recent article in *Current Biology*, Kunda et al. describe a new role for ERM proteins in regulating rearrangements of the cortical cytoskeleton during mitosis.

The precise regulation of cortical tension is essential in dividing cells to allow the complex cell shape changes that accompany cytokinesis. Interphase cells in culture lie flat against the substrate and often have quite irregular shapes, but during mitosis, cells round up and become almost spherical by retracting the cell margin and increasing cortical stiffness (Cramer and Mitchison, 1997). The actin cytoskeleton is crucial to this process, as is myosin II, at least in some cells (Maddox and Burridge, 2003). During interphase, F-actin is found in stress fibers, but as cells enter mitosis, the actin cytoskeleton rearranges to the cell cortex to form a continuous layer of actin filaments positioned beneath the plasma membrane.

While these aspects of mitosis are well known, the underlying mechanisms are poorly understood. Using cultured *Drosophila* S2 cells and RNA interference, Kunda et al. (2008) now demonstrate that Moesin, the only ERM (Ezrin, Radixin, Moesin) protein in *Drosophila,* plays a crucial role in this process. ERM proteins are generally thought to link the plasma membrane to the underlying actin cytoskeleton by interacting with transmembrane proteins via an N-terminal FERM domain (Chishti et al., 1998) and the cytoskeleton via a C-terminal actin-binding domain (Bretscher et al., 2002; Figure 1). Activation of ERM proteins occurs upon phosphorylation of a conserved threonine residue near the C terminus, which unfolds the protein by disrupting interactions between these two domains. In the fly, phosphorylation of this residue is dependent upon Slik, a member of the Sterile-20 family of serine/threonine kinases (Hipfner et al., 2004; Hughes and Fehon, 2006).

To ask if Moesin may be involved in cell-shape changes associated with mitosis, Kunda et al. first examined the activation state of Moesin in interphase and mitotic cells using antibodies specific for activated or phosphorylated Moesin (P-Moesin). P-Moesin is strongly upregulated at the onset of mitosis, initially at the retracting margins then spreading around the entire cortex, and ultimately becomes restricted to the region of the cleavage furrow by telophase. Depletion of either Moesin or the Slik kinase by RNAi has no effect on the morphology of interphase cells but does block retraction of the cell margin and cell rounding in mitotic cells. Conversely, reducing Myosin II activity does not affect either retraction or cell rounding, though these cells exhibit aberrant cortical morphologies. Taken together, these results suggest that both Myosin II and Moesin are necessary for cortical regulation during mitosis, but Moesin alone controls the initial rearrangements of the actin cytoskeleton.

Live imaging of Moesin-depleted cells revealed dynamic defects during mitosis including abnormal contractile waves at the cell cortex and abnormal membrane blebbing, suggesting that some aspect of cortical rigidity may be disrupted. Consistent with this idea, atomic force microscopy showed that although in control cells cortical stiffness increases during the transition from interphase to mitosis, it does not in Moesin-depleted cells. Expression of a phosphomimetic Moesin mutant mimics the increase in cortical rigidity typical of mitotic cells and induces cell rounding, even in the absence of Myosin II function.